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FATE OF FENTHION IN SALT-MARSH ENVIRONMENTS: I. FACTORS AFFECTING BIOTIC AND ABIOTIC DEGRADATION RATES IN WATER AND SEDIMENT

C. R. CRIPE,* E. J. O'NEILL,† M. E. WOODS,‡ W. T. GILLIAM

and P. H. PRITCHARD

United States Environmental Protection Agency, Environmental Research Laboratory,
Gulf Breeze, Florida 32561

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Abstract—Fenthion (Baytex), an organophosphate insecticide, is frequently applied to salt-marsh environments to control mosquitoes. Shake-flask tests were used to study rates of abiotic and biotic degradation of fenthion and the environmental parameters that affect these rates. Water or water-sediment (500 mg dry weight/L) slurries from salt marshes located along the Northwest Florida Gulf Coast were used. Flasks contained 200 µg fenthion/L, and degradation rates were determined by following decrease of fenthion over time. Hydrolysis and biodegradation in water were relatively insignificant fate processes; fenthion disappeared from flasks containing water, formalin-sterilized water, or formalin-sterilized sediment very slowly (half-life ≥ 2 weeks). The presence of nonsterile sediment resulted in a rapid exponential disappearance of fenthion (half-life ≥ 3.8 d). Biodegradation was assumed since sterile sediment systems showed a much slower decrease of fenthion, and the production of polar compounds (hexane-unextractable) from radiolabeled fenthion was greater in the presence of sediment than sterilized sediment. The effect of environmental pH levels (5.0–7.0) on degradation was insignificant. No biotic degradation occurred at low oxygen concentration. An 8°C decrease in incubation temperature decreased the rate of sediment biodegradation 2.5-fold. Light caused a slight, but significant, increase in the biotic and abiotic degradation rates of fenthion in water. A two-fold variation in sediment-mediated degradation was noted among sediment samples from three stations within one field site. Inclusion of whole marsh plants or plant parts increased the disappearance rate of fenthion in test systems.

Keywords—Biodegradation Fate Fenthion Sediment Water

INTRODUCTION

Control of mosquito populations in coastal salt marshes is frequently accomplished by use of pesticides. In the southeastern United States, organophosphate insecticides, such as fenthion (Baytex), naled (Dibrom), chlorpyrifos (Dursban), temephos (Abate), and malathion, are commonly used for this purpose [1,2]. Their usefulness depends on their toxicity to target species and their lack of tox-

icity to nontarget organisms. Since organophosphate insecticides are toxic to a wide range of aquatic invertebrates other than mosquitoes [3–5], adverse environmental effects can be prevented if exposure to the chemical is minimized.

Exposure predictions for organophosphates and other insecticides, however, can be misleading if based on routine tests for persistence in natural waters. Some organophosphates degrade very slowly in water: e.g., parathion hydrolyzes with a half-life of approximately three to four weeks [6]; chlorpyrifos has a half-life of 13 to 120 days in water, depending on temperature and pH [7]. These long half-lives suggest that nontarget species exposure can be significant in aquatic environments. Yet, each of the organophosphates mentioned is considered a safe pesticide in the field, i.e., registration is approved for specified types of application.

Absence of extensive accumulation of organophosphate pesticides in aquatic systems suggests that biotic and abiotic fate processes, either singly

*To whom correspondence may be addressed.

†Technical Resources, Incorporated, c/o United States Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida 32561.

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or in combination, may affect the chemicals. Because salt marshes are shallow, fate processes affected by light, temperature and dissolved oxygen fluctuations, and sediment and plant communities are particularly important. For example, microbial communities associated with lake sediments degrade parathion much more rapidly than do microbial communities in lake water [8]. Hsu and Bartha [9] demonstrated that soil associated with the rhizosphere of plants contained microbial communities that actively degrade parathion. Presence of oxygen may be significant since parathion degrades through different pathways under conditions of low or high oxygen [8,10]. Photodegradation of many organophosphorous pesticides has been observed, including temephos [11], chlorpyrifos [12], and parathion [13]. There is a direct relationship between temperature and both neutral and alkaline hydrolysis rate constants of chlorpyrifos [14].

Information on the biotic and abiotic fate of fenthion is limited, despite its common use for the control of mosquitos in salt-marsh and wetland areas. Preliminary studies in our laboratory indicated a slow loss from water (half-life approximately three weeks). A slow degradation rate, in conjunction with the high toxicity of fenthion to salt-marsh organisms, such as pink shrimp, grass shrimp, and mysids [15], would be cause for concern about the toxic effect of this organophosphate on nontarget species. Consequently, more information about the factors that control the biotic and abiotic degradation of fenthion in salt-marsh environments is required before conclusions about the potential for harmful effects on an aquatic ecosystem can be assessed.

We report here the effect of sediments, plants, temperature, oxygen, light, pH, and inoculum source on the biotic and abiotic degradation of fenthion in shake-flask tests. Our results indicate that some of these factors, especially presence of sediment, greatly affect degradation of fenthion in aquatic systems.

MATERIALS AND METHODS

All environmental samples were taken from three salt marshes on Santa Rosa Island between Pensacola Beach and Ft. Walton Beach, Florida. Range Point salt marsh is predominantly a *Spartina alterniflora* marsh, approximately 0.5 m deep, and slightly higher in salinity than the other sites. Site A-10 is a small embayment surrounded by *Juncus* and ringed by *Spartina* plants. The site has a maximum depth of 1 m and flushes more completely than Range Point because of a wider opening to

Santa Rosa Sound. Site SA-79 is a shallow (0.5 m) *Juncus roemerianus* salt marsh with a narrow opening to Santa Rosa Sound; flushing with the sound is greatly restricted. The three sampling stations at this site are located at the north and south ends of and in the middle of the marsh.

Spartina plants were collected at site A-10. Water was collected below the surface at each site in a solvent-rinsed carboy and filtered to 3 μm prior to use. Sediment samples were collected by scooping sand and detritus from the sediment bed surface into a flask. Sand was removed by differential settling and the remaining highly organic detrital fraction was passed through a 2-mm mesh screen. The sediment concentration of the slurry was determined by filtering 5-ml subsamples onto a pre-dried 0.45- μm membrane filter and drying. Both site water and sediment slurry were maintained in the laboratory by stirring at room temperature for 24 to 48 h before starting a test.

Degradation studies of fenthion (O,O-dimethyl-O-[4-(methylthio)-*m*-tolyl]-phosphorothioate) were performed by using shake-flask tests [16]. Fenthion was obtained from the U.S. Environmental Protection Agency, Reference Standards Repository, Research Triangle Park, North Carolina. A specified quantity of fenthion in hexane carrier was placed in the bottom of a 4-liter glass bottle and the hexane evaporated under nitrogen. Seawater from the appropriate sampling site was added to produce a nominal fenthion concentration of approximately 300–400 $\mu\text{g/L}$. The solution was stirred and sampled until a constant concentration was reached. One liter of the resulting fenthion solution was diluted with site water to produce 200 $\mu\text{g/L}$ in each 2-liter test flask. When the effect of sediment concentration was examined, sediment slurry was added to flasks to give concentrations of 500, 1,000, 2,500 and 5,000 mg/L. Water and sediment flasks without fenthion also were prepared to monitor for extraneous naturally occurring organics that might interfere with gas chromatographic analysis of the fenthion. Sterile water and sterile sediment slurry flasks were prepared with 20 ml formalin per liter. Flasks were incubated on a shake table at 140 rpm in the dark at 25°C. When temperature effects were studied, a refrigerated circulating water bath was used to maintain subambient temperature in the incubator. All flasks were checked every other day for changes in pH; when necessary, pH was adjusted to the starting value (± 0.2 units) with HCl or NaOH.

Decreases in fenthion concentration with time were determined by placing 10 ml samples of water or sediment slurry from a flask in 50-ml glass

tubes containing 10-ml hexane, capping with Teflon-lined screw caps and shaking on a rotator (60 rpm) for 1 min. Following centrifugation to break up emulsions, a subsample of the hexane extract was injected into a gas chromatograph (Model 5842, Hewlett-Packard Co., Palo Alto, CA) fitted with a nitrogen-phosphorous detector and a column (1.8 m long, 2 mm i.d.) packed with 3% OV-101 or a mixture of 0.75% SP-2250 and 0.97% SP-2401.

A SAS (Statistical Analysis System, SAS Institute, Cary, NC) regression analysis was used to determine a first-order rate constant, K_1 , using the following regression model:

$$\ln C = a + K_1 t \quad (1)$$

where C is the concentration of test compound ($\mu\text{g/L}$), a is the y-axis intercept, and t is time. The half-life (T) can then be defined:

$$T = \frac{0.693}{K_1} \quad (2)$$

The F statistic was used to test the significance of the regression. A SAS general linear model procedure was used to compare the slopes of two or more tests. A significance level of 0.01 was used for both statistical tests [16]. With this value, selected through previous shake-flask tests, we can obtain a conservative estimate of potential hazard by reducing the likelihood of incorrectly concluding that a test compound has a degradation rate different from zero. Use of the 0.01 level of significance also minimizes instances in which half-life differences of only a few hours or days are significantly different.

Abiotic and biotic degradation of fenthion in settled sediments was determined in beakers that contained 500 mg sediment, 1 liter water, and 200 μg fenthion with or without 20 ml formalin. To avoid possible stratification of fenthion, a glass rod attached to a 300 rpm motor was used to stir the water column continuously without suspending the sediment.

The effect of low-oxygen conditions on degradation of fenthion was determined by using the shake-flask degradation test, except that oxygen was purged with nitrogen gas. Flasks were stoppered to prevent reentry of oxygen during incubation on the rotary shaker and flasks were purged after each sampling.

Biodegradation experiments with sediment and radiolabeled substrate (ring1- ^{14}C) fenthion, 18.7 mCi/mM; Mobay Chemical Corporation, Kansas

City, MO) used a nominal concentration of 3×10^6 dpm of radioactivity per liter, approximately 10% of the total fenthion concentration (220 $\mu\text{g/L}$) in the flasks. Radioactive fenthion was added only to the sterile and nonsterile sediment flasks, each of which contained 5,000 mg sediment/L. Samples were analyzed periodically to determine fenthion and radioactivity in various components of the system. Duplicate 3-ml samples of sediment-water slurry from the flasks were placed in scintillation cocktail (PCS, Amersham Corp., Arlington Heights, IL) and total radioactivity measured, using a scintillation counter (Model LS8200, Beckman Instruments, Inc., Fullerton, CA). Duplicate 20-ml samples were centrifuged to pellet the suspended sediment and a 3-ml sample supernatant was removed for scintillation counting. Ten milliliters supernatant was extracted with 10 ml hexane and the hexane analyzed for residual fenthion (GC) and total radioactivity. Three milliliters extracted water was also counted. The remaining supernatant was discarded and the sediment pellet was extracted with 5 ml acetonitrile. Sediment was removed again by centrifugation and a 2-ml subsample of the acetonitrile extract was removed for radioanalysis. An additional 2 ml of the acetonitrile extract was extracted with 4 ml hexane and 6 ml 2% aqueous sodium sulfate, and radioactivity in the hexane extracts was measured. After a second acetonitrile extraction and drying, the sediment was combusted (Tri-carb Oxidizer, Packard Instruments Co., Inc., United Technologies, Downers Grove, IL) to determine nonextractable (bound) radioactivity.

Radiolabeled carbon dioxide production, an indicator of biodegradation, was determined by continuously purging the headspace of flasks that contained [^{14}C]fenthion with air and trapping the carbon dioxide in Carbosorb (Packard Instruments). An equal volume of Permafluor-IV cocktail (Packard Instruments) was added to the Carbosorb prior to radioanalysis.

The effect of pH on the abiotic degradation of fenthion in sediment slurries was determined by suspending formalin-sterilized sediments in buffered seawater (0.01 M potassium phosphate for pH 6 and 7; 0.01 M potassium hydrogen phthalate for pH 5.0), adding radiolabeled fenthion (nominally 3×10^6 dpm per liter) and unlabeled fenthion to a final concentration of 200 $\mu\text{g/L}$, then sampling as in the biodegradation experiments. Samples were tested for sterility using standard agar plating techniques.

Effect of clay on abiotic fenthion degradation was determined in flasks that contained 500 mg

sediment/L plus either 7.5, 75 or 750 mg montmorillonite clay/L, equivalent to 1, 10 and 100 times the clay content (approximately 1.5%) of the 500 mg of sediment. All flasks contained formalin and were buffered at pH 7.0. The starting concentration of fenthion in each flask was 200 $\mu\text{g/L}$.

Effect of a salt-marsh plant, *Spartina alterniflora*, on degradation was determined in flasks that contained 1 liter of fenthion solution (200 $\mu\text{g/L}$) and either a whole plant (leaves and roots), plant parts (i.e., outside leaves, inside leaves), or 500 mg (dry weight) of sediment that surrounded the plant roots. After sediment around the roots was removed with site water, the root system was cut from the stem of the plant, and four outside leaves were gently peeled from the remaining stem. All severed areas were dipped in molten paraffin to prevent leaching of organic materials. Plant material was prepared for residual fenthion analysis by cutting the plant parts into 2-mm pieces, suspending them in twice their weight of water and grinding with a Polytron (Brinkman Instruments, Westburg, NY). A 10-g portion of the resulting slurry was mixed with 10 ml acetonitrile, reground and centrifuged. The supernatant was removed and the pellet extracted twice more with 5-ml volumes of acetonitrile. Extracts were combined, added to 70 ml of aqueous 2% sodium sulfate solution and extracted three times with 10-ml hexane. The hexane extracts were combined and concentrated to 25 ml under a stream of nitrogen prior to quantification by gas chromatography.

We examined the effect of laboratory lighting and ambient sunlight on the degradation of fenthion in straight-sided glass reaction kettles (Corning Glass Co., Corning, NY) covered with plexiglass lids. Each system contained 200 μg fenthion per liter of unfiltered water. Sterile (formalin-amended) and nonsterile treatments were tested under both light and dark conditions at 20°C in a constant temperature bath. Black paint and aluminum foil were used to exclude light in the dark treatment. Each system was stirred with bent glass rods rotating at 300 rpm. Samples were periodically removed for extraction and quantification. Two treatments (light and dark, both nonsterile) were tested outdoors and kept unstirred in a water bath at ambient temperature.

RESULTS

Figure 1 shows the disappearance of fenthion in sterile and nonsterile seawater from site A-10 with and without sediment. Degradation rate constants and half-lives are listed for this and other sites in Table 1. The presence of nonsterile sediment in the flasks resulted in the most rapid loss of fenthion. All disappearance curves with sediment appeared to be first-order, except for a test conducted during August 1984 at site SA-79 where 500 mg nonsterile sediment produced a decay curve characteristic of adaptation after 100 h. By "adaptation" we are referring to the phenomenon where a compound is degraded more rapidly by a microbial population in a test system previously exposed to the com-

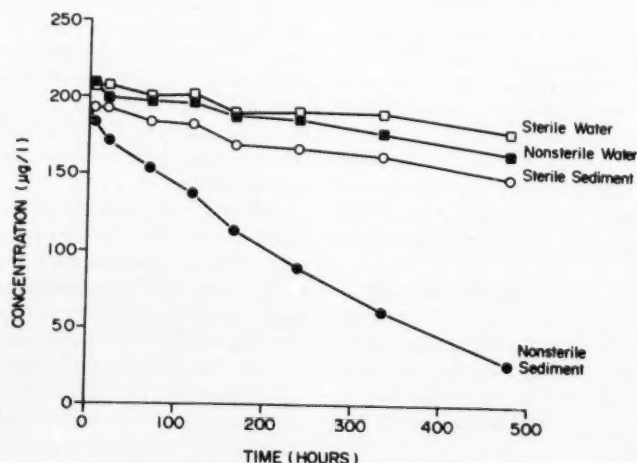


Fig. 1. Disappearance curves for fenthion in water and water with 500 mg sediment/L under sterile and nonsterile conditions.

Table 1. Degradation of fenthion in shake flasks containing water or water and sediment from several salt-marsh sites and incubated under sterile (formalin-amended) and nonsterile conditions

Site	Date	Treatment	Milligram sediment/L	First-order rate constant (h^{-1})	Half-life (d)	r^2	Significance ^a
RP	10/82	Nonsterile	0	-0.0013	22	0.71	0.0169
RP	10/82	Nonsterile	500	-0.0075	3.8	0.99	0.0001
RP	10/82	Sterile	500	-0.0020	14	0.80	0.0164
A-10	7/84	Nonsterile	0	-0.00047	>30	0.97	0.0001
A-10	7/84	Nonsterile	500	-0.0039	7.4	0.98	0.0001
A-10	7/84	Sterile	0	-0.00031	>30	0.92	0.0001
A-10	7/84	Sterile	500	-0.00058	>30	0.95	0.0001
SA-79	8/84	Nonsterile	0	-0.0021	14	0.91	0.0002
SA-79	8/84	Nonsterile	500	-0.0028 ^b	10	0.94	0.0308
SA-79	8/84	Nonsterile	1,000	-0.0064	4.5	0.98	0.0011
SA-79	8/84	Nonsterile	2,500	-0.014	2.1	0.98	0.0002
SA-79	8/84	Nonsterile	5,000	-0.016	1.8	1.00	0.0001
SA-79	8/84	Sterile	0	-0.00012	>30	0.27	0.1887
SA-79	8/84	Sterile	500	-0.00061	>30	0.73	0.0067
SA-79	8/84	Sterile	5,000	-0.0014	21	0.83	0.0003
SA-79(South)	12/83	Nonsterile	500	-0.0026	11	0.97	0.0001
SA-79(Middle)	12/83	Nonsterile	500	-0.0025	12	0.95	0.0001
SA-79(North)	12/83	Nonsterile	500	-0.0044	6.6	0.98	0.0001

^aLevel of significance of the regression.^bPrior to "adaptation."

pound [17]. Respiring the flasks with fenthion (200 $\mu\text{g/L}$, nominal concentration) resulted in a first-order rate approximately 10-fold greater than the slope observed during the first 100 h. However, later experiments (P. Lefcourt, U.S. EPA Environmental Research Laboratory, Gulf Breeze, FL, personal communication) with the same sediment slurry, as well as sediment from the Range Point test site, did not indicate either adaptation, the enrichment of fenthion-degrading bacteria, or the enhanced degradation of another organophosphate (methyl parathion).

Disappearance of fenthion also occurred in other treatments. Most rate constants were significant ($p < 0.01$), as indicated in Table 1. A comparison of rate constants of the water treatments (sterile and nonsterile) indicated a small but significant difference in samples from both A-10 and SA-79, suggesting that microbes in the water were degrading fenthion. Differences between sterile treatments (water and sediment) at both sites also suggested that the presence of sterile sediment enhances fenthion degradation.

Results from experiments with radiolabeled substrate (Fig. 2) showed that disappearance of hexane-extractable radioactivity (largely fenthion) in the nonsterile flask that contained 5,000 mg sediment/L coincided with a proportional increase in hexane-unextractable radioactivity (polar degrada-

tion products). Very small quantities of radiolabeled carbon dioxide (< 2% of initial radioactivity) were detected in the sterile and nonsterile sediment flasks. The radioactivity trapped as carbon dioxide from the sterile flask was subtracted from that of the nonsterile flask, resulting in less than 1% mineralization. Nonextractable (bound) residues associated with the sediment amounted to 9.4% of the initial radioactivity in the nonsterile flask and 4.6% in the sterile flask.

Rates for fenthion biodegradation in sediment slurries (500 mg/L) taken from three different sites and from three different stations in the SA-79 site are shown in Table 1. Rate differences were as much as threefold. Fenthion degradation rates in nonsterile sediments (Fig. 3) were proportional to sediment concentrations of up to 2,500 mg/L (second-order rate constant, $0.0048 \text{ h}^{-1} \text{ g}^{-1}$). Two volumes of well-mixed sediment slurry (500 and 5,000 mg, dry weight) were allowed to settle overnight in cylinders, and the supernatant liquids were used in a shake-flask test to determine if observed biological activity associated with sediment slurries was actually associated with particulates. These water extracts did not significantly ($p > 0.30$) stimulate the biodegradation rate of fenthion over that of water (-0.00050 and -0.0013 h^{-1} for 500 and 5,000 mg sediment/L versus -0.00088 h^{-1} for nonsterile water).

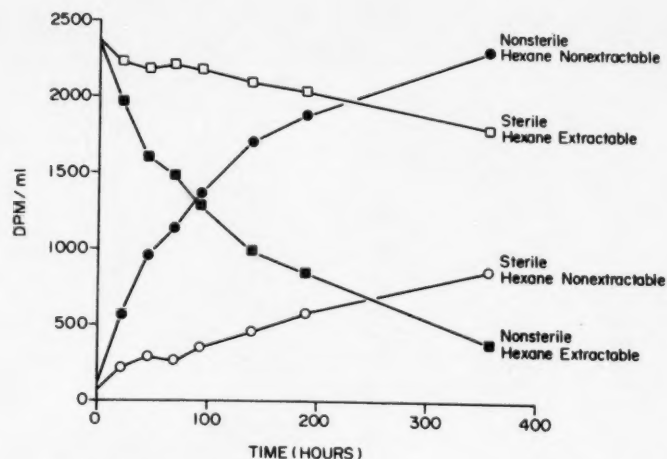


Fig. 2. Hexane-extractable (largely fenthion) and -nonextractable (polar degradation products) radioactivity from sterile and nonsterile flasks containing 5,000 mg sediment/L of water. Radioactivity represents the sum of separate extractions on water and sediment fractions.

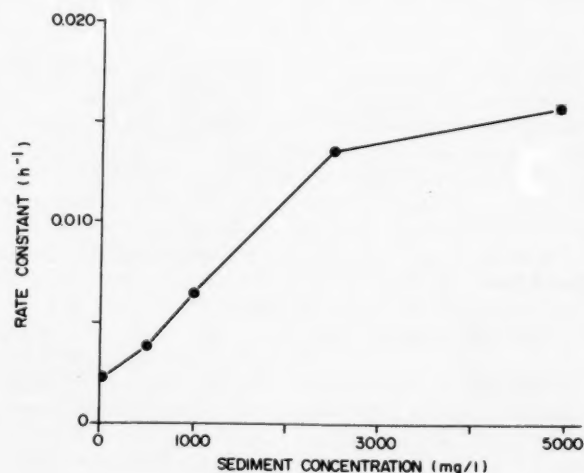


Fig. 3. First-order rate constants of fenthion degradation at four nonsterile sediment concentrations.

Sediment-enhanced biodegradation rates also were affected by physical treatment of the sediments and by low-oxygen conditions. Nonsterile suspended sediments (500 mg/L) resulted in a significantly faster ($p = 0.0006$) degradation rate of fenthion ($-0.0090 h^{-1}$) than did the same amount of settled sediments ($-0.0062 h^{-1}$). Incubation of sediment-water slurries under low-oxygen conditions (aeration with nitrogen) eliminated biological

degradation; both sterile sediment and low-oxygen nonsterile flasks had the same rate: $-0.0013 h^{-1}$.

Abiotic degradation was proportional ($r^2 = 0.99$) to a 10-fold sediment concentration range with a second-order rate constant (rate/g sediment) of $-0.00023 h^{-1}g^{-1}$ (Fig. 4). At 500 mg sediment/L, rates were the same for both suspended and settled tests ($-0.0013 h^{-1}$). The presence of a 100-fold range in clay concentration in flasks that

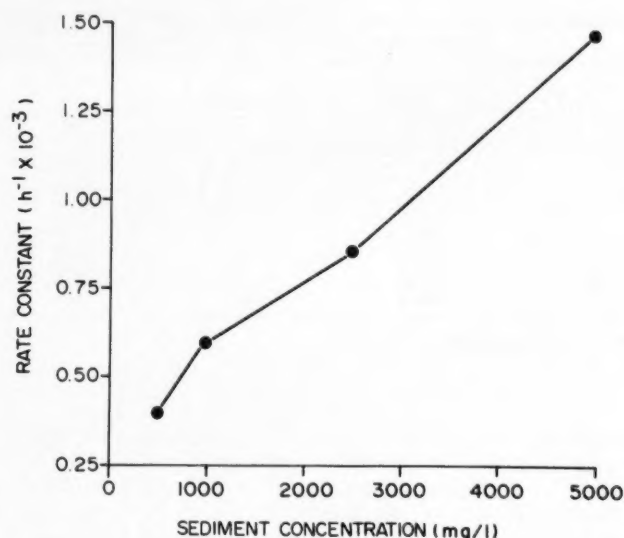


Fig. 4. First-order rate constants of fenthion degradation at four sterile sediment concentrations.

Table 2. Degradation rates of fenthion in unfiltered seawater from site A-10 under different lighting conditions

Treatment	Lighting condition	First-order rate constant (h ⁻¹)	r ²	Significance ^a
Sterile	Fluorescent	-0.00083	0.93	0.0001
Sterile	Dark	-0.00011	0.39	0.0719
Nonsterile	Fluorescent	-0.0020	1.00	0.0001
Nonsterile	Dark	-0.00072	0.91	0.0001
Nonsterile	Sunlight ^b	-0.0046	1.00	0.0001
Nonsterile	Dark ⁿ	-0.0016	0.98	0.0001

^aLevel of significance of the regression.

^bDenotes tests that took place outside of the laboratory and subject to environmental temperature fluctuations.

contained 500 mg sediment/L had no effect on abiotic degradation rates (-0.00038 to -0.00040 h⁻¹).

Light and temperature significantly affected fenthion degradation, but pH did not. Acidic to neutral pH values (5.0–7.0) all produced essentially the same abiotic degradation rates (-0.00040 to -0.00047 h⁻¹) in the presence of sediment. Degradation in a nonsterile flask containing 500 mg sediment/L was 2.5-fold greater at 23°C than at 15°C (-0.0061 h⁻¹ versus -0.0024 h⁻¹). Sterile and nonsterile water systems exposed to laboratory (fluorescent) lighting degraded fenthion at significantly greater rates than did their counterparts in-

cubated in the dark (Table 2). A similar difference occurred in the presence and absence of sunlight. Warmer temperatures probably accounted, in part, for the elevated rates in the outdoor systems. Photolytically enhanced biodegradation also is indicated in the nonsterile fluorescent-lighted treatment, because the rate in this treatment is somewhat greater than the sum of the rates for the nonsterile, dark treatment and the sterile, light treatment.

The presence of *Spartina* plants or plant parts in flasks greatly increased the rate constant for fenthion disappearance in nonsterile water (Table 3). Similarly, very rapid losses were also observed with whole *Juncus roemerianus* plants (data not shown).

Table 3. Degradation rates of fenthion in water containing plants (*Spartina alterniflora*), plant parts or sediment from around the roots of plants

Type	First-order rate constant (h ⁻¹)	Half-life (d)	r ²	Significance ^a
Whole plant	-0.085	0.34	0.99	0.0002
	-0.039	0.74	0.93	0.0087
Roots	-0.053	0.54	0.92	0.0091
Outside leaves	-0.011	2.6	0.94	0.0001
Inside leaves	-0.0014	21	0.48	0.0185
Sediment around roots	-0.0021	14	0.97	0.0022
Nonsterile water	-0.00052	>30	0.80	0.0162

^aLevel of significance of the regression.

Fenthion disappearance also occurred in the presence of *Spartina* outer leaves and to a lesser extent in the presence of inner leaves. The increase due to whole plant parts or plant roots was comparable to the addition of 500 mg sediment (see Table 1). Residue analysis indicated no significant fenthion accumulation in any of the whole plants or plant parts.

DISCUSSION

In sterile water, fenthion disappeared at a slow rate significantly different from zero (see Table 1) at site A-10 but not at SA-79. Loss in sterile water is probably the result of hydrolysis. Our rates are similar to the 90-d (± 20 d) half-life observed by Wolfe (U.S. EPA Environmental Research Laboratory, Athens, GA, personal communication) for the neutral hydrolysis of fenthion in distilled water. Disappearance in our systems cannot be attributed to a photolysis (flasks were incubated in the dark) or a formalin effect (rates were not different from filter-sterilized seawater). We conclude, therefore, that hydrolysis of fenthion in seawater of salt-marsh areas would be of minor consequence.

Sediments enhanced abiotic degradation. Macalady and Wolfe [18] examined the effect of sediment on the hydrolysis of chlorpyrifos and found that rates of neutral hydrolysis were not affected by sorption to sediment, while alkaline hydrolysis rates were substantially reduced by sediment sorption. We found that abiotic fenthion degradation rates were increased by the presence of 500 mg sediment per liter of water. Enhancement was not the result of improperly sterilized sediments, since viable bacteria were not detected on agar plates. An interactive formalin-sediment effect, however, cannot be ruled out even though we believe that it is unlikely from a chemical reactivity standpoint. Wolfe (personal communication) has shown a sim-

ilar enhancement effect in formalin-sterilized river sediments with carboxylic acid esters (i.e., 2,4-D) but not with organophosphate pesticides. He suggests that the enhancement may be due to the presence of extracellular enzymes on the sediment surface since autoclaving destroyed the activity. Mulla et al. [19], on the other hand, reported that clay minerals, especially kaolinite, promoted the hydrolysis of parathion under laboratory conditions. We found that clay minerals, with surfaces that are unlikely to harbor enzymatic activities, did not significantly stimulate the hydrolysis of fenthion; thus, the effect of sediment on hydrolysis may not be the result of a surface interaction. The insignificant effect of pH below 7.0 further suggests that acidic conditions of a sediment bed do not affect hydrolysis rates. This agrees with the generalization that organophosphates that are most stable at pH 1-5, but hydrolysis rates increase steeply at pH values above 7 or 8 [20]. Under the relatively well-buffered conditions of a salt marsh, pH should not be a significant factor in fenthion degradation.

The more rapid disappearance of fenthion in the nonsterile water treatment compared to the sterile water treatment suggests that some form of biological degradation occurred since no other loss mechanism would account for this disappearance. This is presumably the result of nonspecific biological activity, such as the elaboration of extracellular esterases, rather than the direct metabolism of the pesticide for carbon and energy by microbial communities. Similar hydrolytic enzymes in pure or mixed cultures or soil extracts have been demonstrated for parathion, chlorpyrifos, and other organophosphates [21]. We have been unable to isolate bacteria that would use fenthion as a carbon source for growth, but we have obtained pure cultures of bacteria, randomly selected from agar

plates used to measure total heterotrophs that, when grown in a minimal salts medium containing succinate, will promote the disappearance of fenthion. This procedure is analogous to that used by Lewis and Holm [22] to quantitate the number of methyl parathion degraders in river water. In both cases, the disappearance of the pesticides was assumed to be the result of extracellular esterase activity.

The relatively slow degradation rate observed in nonsterile seawater suggests that the biomass of bacteria capable of transforming fenthion was very low in estuarine water samples we tested. This relatively long half-life (14 d or more) for fenthion by itself would cause concern for significant exposure to nontarget organisms. Eichelberger and Lichtenberg [23], however, reported a shorter half-life (7 d) for fenthion in fresh water systems. This may have been the result of substantially higher microbial biomasses in their water samples.

The stimulatory effect of sediment on the degradation of fenthion in water is an environmentally relevant observation because of its potential importance in predicting concentrations in estuarine waters following pesticide applications. Sediment-enhanced biodegradation, i.e., more rapid loss of fenthion from water when nonsterile sediments are present, has been observed with methyl parathion [24] as well as other industrial organic compounds such as dibutylphthalate [25], mono-, di- and triethylene glycols [26], *p*-chlorophenol, trichlorophenol, trichlorophenoxyacetic acid, chlorobenzene [27] and *p*-nitrophenol [24]. With fenthion, the addition of 500 mg sediment per liter more than doubled the degradation rate constant of water alone. No one as yet has adequately explained the mechanism behind this enhancement, but it may simply be due to a larger biomass associated with the sediment relative to the water column.

Sediment appears to enhance transformation of the parent chemical by biological processes rather than simple sequestering of the chemical on the sediment surface in a manner that precludes detection by our analytical methods. Sequestering also would be expected to occur in sterilized sediment, which did not happen in our studies. In addition, considerably more transformation (polar) products, as suggested by the formation of radioactive materials unextractable by analytical procedures normally sufficient to extract fenthion, were formed in a nonsterile sediment system. Although we have no confirmatory data, these operationally unextractable (polar) products could include the esterase hydrolysis product, thiomethyl cresol. Finally,

larger amounts of radioactivity unextractable with acetonitrile remained associated with nonsterile sediment rather than with sterile sediments. These so-called "bound residues" have been observed by several investigators working with organophosphate insecticides in soil [28,29] and in sediments [8,24], and their presence has been suggested as evidence for biodegradation. Saxena and Bartha [30] have presented evidence to suggest that bound residues of aniline herbicides in soil are the result of biologically mediated covalent binding of the parent chemical or one of its degradation products to humic acid moieties. Others have shown that with the proper extraction technique (e.g., solvent extraction after 6 N HCl acid treatment), degradation products can be recovered from the bound residue fraction [31]. Bound residues observed in our experiments, therefore, are probably also the consequence of some biodegradation event.

The importance of sediment-enhanced degradation in estuarine environments will depend on the probability of fenthion reaching the sediment bed or the possibility of sediments being suspended in the water column. Both conditions are possible in salt marshes where fenthion might be used for mosquito control; wind-driven water turbulence in shallow bodies of water commonly increases the movement of water entering the sediment bed. Fenthion has an octanol/water partition coefficient ($\log_{10}$) of approximately 3.7 (J. Moore, U.S. EPA Environmental Research Laboratory, Gulf Breeze, FL, personal communication); consequently the pesticide will have a significant affinity for the organic matter in the sediment bed. However, water movement, alone, through the very porous top layer of the sediment bed (less than 1.0 cm thick) may provide sufficient exposure to allow the pesticide to be degraded.

To accurately predict field concentrations of fenthion after application it is important to know the relationships among the rate of fenthion degradation and the amount and metabolic state of the sediment particles. We found the degradation rate to be proportional to organic sediment (detritus) concentrations up to 2,500 mg/L, but not at 5,000 mg/L. Thus, organic sediment has about the same metabolic activity per unit mass. Degradation with depth or surface area of the sediment bed can therefore be predicted, knowing the distribution of organic detritus in the bed. In our particular salt-marsh study sites, the organic detritus is mixed with sand which is generally easy to separate from the detrital component. In areas with different sediment compositions, it might be better to normalize

the degradation against the total organic carbon content of each bed layer. The observed deviation from linearity at the high sediment concentration, a concentration that is typical of some salt-marsh areas, makes extrapolation of the laboratory data to the field more difficult. There are several possible explanations for the deviation. Slower degradation rates at the higher sediment concentration may have been the result of physical factors because this large amount of particulate material was difficult to keep uniformly suspended in the shake flask. If a xenobiotic compound that is sorbed to the sediment surface is unavailable for degradation [32-34], then enough fenthion may be sorbed at the highest sediment concentration to slow the degradation rate. Based on this theory, however, any amount of chemical sorption to the sediment surface should reduce degradation rate constants; yet, in our experiments, a change in rate was not observed until the sediment concentration fraction reached 5,000 mg/L, where theoretically 75% of the added fenthion was sorbed. The high sediment concentrations may also have made nutrients, dissolved oxygen, or both less available per sediment particle. Thus, in a salt marsh we would expect the presence of sediments to significantly reduce water column concentrations of fenthion, both by partitioning and biodegradation.

Our studies also show that once fenthion gets into the sediment bed, it will be degraded at a somewhat slower rate than that determined in the shake-flask tests, i.e., settled sediments appear to have less effect than suspended sediments. These results suggest that not enough bacteria were released from the sediment during agitation to stimulate degradation significantly. We predict most of the degradation in the sediment bed will occur as a result of the bacteria associated with the sediment surface rather than the bacteria associated with the interstitial water. Finally, at depths where low-oxygen conditions are encountered in the sediment bed, we would expect very little degradation to occur. For most salt marshes, anaerobic conditions will exist very close to the sediment surface and, thus, we would predict that fenthion would be degraded in the top 1 cm or less of the sediment bed.

Several other factors also can affect the fate of fenthion in salt-marsh environments. The presence of sunlight or artificial light significantly stimulated fenthion degradation (Table 2). Light, especially sunlight, is known to accelerate transformations (photooxidation, photoisomerization, hydrolysis) of organophosphorous compounds [20]. In our

studies, exposure to sunlight produced a decay rate constant greater than that observed under fluorescent lighting. This was at least partly due to the higher intensity and different spectral quality of sunlight, but also was probably related to the increased outdoor temperature, which reached 35°C or higher during the day. Live bacteria or algal cells present possibly acted as photosensitizers; accelerated photolysis of organophosphorous compounds by algal cultures, both living and heat killed, was demonstrated by Zepp and Schlotzhauer [35]. Sethunathan et al. [36] reviewed several other studies that reported algal acceleration of parathion degradation. In a salt marsh, photolysis of fenthion in water and perhaps on the upper sediment surface should be increased by the shallow water. These processes are likely to be significant in salt marshes, with their relatively large suspended and benthic algal biomass, due to high nutrient concentrations and high light intensity.

A temperature increase of approximately 8°C produced a 2.5-fold increase in the sediment-mediated biodegradation rate of fenthion. Temperature fluctuations in salt-marsh sediments due to ambient temperature changes, solar energy adsorption, and biological activity would be expected to cause significant variations in biodegradation rates.

Spatial and temporal variability in degradation rate constants have not been extensively reported. Paris et al. [37] indicated that degradation in water could be normalized for total numbers of viable bacteria at more than 30 diverse sites for compounds such as malathion, chloropham, and the butoxyethyl ester of 2,4-dichlorophenoxyacetic acid. Studies in our laboratory have shown that sediment-enhanced (500 mg/L) degradation of methyl parathion was independent of total numbers of viable bacteria, but varied only fivefold over a three-year period at 13 sites [38]. The three-fold variability that we observed with fenthion indicates, that, like methyl parathion, a relatively low spatial variability in biodegradation rates may exist in estuarine areas we examined.

The higher degradation rate constants in the presence of *Spartina* plants or plant parts were probably due to either the activities of bacteria associated with the surface of the plant or uptake and degradation by the plant tissues. Our observation of larger microbial communities on the surface of outer leaves, as opposed to the inner leaves, supports the former suggestion. If the plants were actively involved in the uptake, it is likely that they also metabolized the fenthion because very little parent compound was found following residue analysis.

Fenthion metabolism in plants has been reported [39]. Regardless of the mechanism, plants could be an additional factor in the overall fate and biodegradation of fenthion in salt-marsh environments.

Although the rhizosphere soil of some plants has also been shown to enhance organophosphate-degrading activity [9,40], sediment from around the roots of *Spartina* that we tested showed somewhat lower activity (Table 3) than sediment taken a few meters from the plant roots (Site A-10, Table 1). This difference appears to be consistent with the station-to-station variability.

In summary, although fenthion is relatively persistent in water due to low biotic and abiotic degradation rates, our studies indicate that several environmental factors present in salt marshes could potentially increase degradation rates under actual field conditions. Frequent contact with sediment and plants, higher temperatures and direct sunlight are probably the most important factors. Further studies at our laboratory with the mathematical modeling of microcosms containing intact sediment cores provides a mechanism for determining the relative significance of these factors [41]. The environmental hazard posed by the application of fenthion to a particular salt marsh would depend on the integration of these fate processes with toxicological information on sensitive nontarget species.

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